Clinical Characterization of Polymorphisms in the Sulphonylurea Receptor 1 Gene in Japanese Subjects with Type 2 Diabetes Mellitus

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To assess the association of polymorphisms at the sulphonylurea receptor (SUR1) gene with the development of Type 2 diabetes mellitus, 456 subjects, 236 with Type 2 diabetes and 220 non-diabetic controls, were analysed for variants at exon 7, exon 22 and intron 24 of the SUR1 gene by the polymerase chain reaction and restriction fragment length polymorphism. The T761T substitution in exon 22 of the SUR1 gene was not found in either diabetic patients or non-diabetic controls. Both the exon 7 variant and the intron 24 variant were present in both groups at similar frequencies. No significant association was seen between either variant and obesity. Diabetic patients homozygous for the -3C allele of intron 24 had a higher ratio of positive family history than patients homozygous for the -3T allele ($p = 0.03$). We conclude that these polymorphisms are not major determinants of diabetes and obesity in the Japanese population. © **1998 John Wiley & Sons, Ltd.**

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Introduction

Both genetic and environmental factors contribute to susceptibility to Type 2 diabetes mellitus. However, the major genes associated with the common forms of Type 2 diabetes have not yet been identified. The polygenic disease is characterized by impaired insulin secretion and insulin resistance. Genes involved in stimulus-secretion coupling of insulin may be good candidates for susceptibility genes.

The closing of adenosine-5′-triphosphate-sensitive channels (K_{ATP} channels) in beta cells is a key step in glucose-induced insulin exocytosis.[1–3] The beta-cell K_{ATP} channel is a complex of an inwardly rectifying K_{ATP} channel subunit (Kir6.2) and a sulphonylurea receptor (SUR1).[4,5] Sulphonylurea derivatives exert their hypoglycaemic effect by closing the K_{ATP} channel, which promotes an influx of calcium with subsequent stimulation of insulin release.[6] Mutations in the SUR1 gene have been found to be responsible for familial persistent hyperinsulinaemic hypoglycaemia of infancy,[7] showing that SUR1 protein plays a regulatory role in the control of insulin secretion.

Recently an association of polymorphisms at the SUR1 gene with Type 2 diabetes and obesity was shown in Caucasian populations.[8,9] A silent mutation (C→T, T761T) in exon 22 and a nucleotide substitution (T→C) at −3 of intron 24 were more common in patients with Type 2 diabetes than in non-diabetic controls in UK and American white populations, while a missense mutation (T→G, S1370A) in exon 7 showed equal frequency in patients with diabetes and controls.[8] The frequency of the exon 22 variant was higher in obese subjects in French Caucasians.[9] The Japanese are genetically susceptible to Type 2 diabetes but heterogeneity has been shown in the association of Type 2 diabetes with several candidate genes between Japanese and Caucasians. No linkage was reported between microsatellite markers near the SUR1 gene and Type 2 diabetes in Japanese.[10] In this study we analysed the association of polymorphisms in the SUR1 gene with diabetes and obesity in the Japanese population.

Patients and Methods

Subjects were recruited for the study from individuals who attended the Kumamoto Red Cross Health Care Center for health screening and gave informed consent, and patients with Type 2 diabetes treated at Kurume University Hospital (Table 1). A total of 456 subjects, 236 with Type 2 diabetes and 220 non-diabetic controls,

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were investigated for variants of the SUR1 gene. Diabetes was diagnosed according to the following criteria: basal plasma glucose concentration more than 7.8 mmol l^{-1} on two occasions or glucose higher than 11.1 mmol l^{-1} at 120 min after a 75-g oral glucose load. Age-matched non-diabetic controls were selected on the basis of a plasma glucose concentration less than 7.8 mmol l^{-1} at 120 min after a 75-g glucose load.

Genomic DNA was extracted from total blood by the phenol/chloroform method following incubation with proteinase K (Sigma, St Louis, Mo., USA). Amplification of the SUR1 gene sequences was performed by polymerase chain reaction (PCR) in a volume of 10 μl containing 0.1 U of AmpliTag Gold™ (Takara, Otsu, Japan), 10 mmol l^{-1} Tris-HCl pH 8.3, 50 mmol l^{-1} of KCl, 1 mmol l^{-1} $MgCl_2$ (1.5 mmol l^{-1} $MgCl_2$ for exon 7), 100 μmol l^{-1} of dNTPs (Takara) with the following primers: for exon 22, 5′-AGAGCTCAGTATGCCTTTCC-3′ (forward) and 5′-GGTGATGTGGCTCCCTTGG-3′ (reverse); for exon 7, 5′-AGGGAGAGGGGTGGGAA-GAGTCCAA-3′ (forward) and 5′-CGTGCACTGACT-TCTGTCCAGGGG-3′ (reverse); and for intron 24, 5′-CCCGGCCCCACTCACATCTG-3′ (forward) and 5′GGAGGATGGTTAAAAGGAGATT-3′ (reverse). Cycles consisted of an initial denaturation at 95°C for 9 min and 35 cycles of denaturation of 95°C, annealing at 55°C for exon 22 and intron 24, or at 66°C for exon 7, and extension at 72°C for 1 min each. PCR products were analysed on 2 % agarose gels to confirm the proper amplification. Then the amplified PCR products were subjected to enzymatic digestion with Bsa01 for exon 22, Mwo 1 for exon 7, or Pst 1 for intron 24. After an incubation with Bsa01 or Pst1 at 37°C or with Mwo1 at 60°C for 3 h, the digested samples were separated by electrophoresis through agarose gel and visualized by staining with ethidium bromide. The exon 22 and intron 24 substitutions abolish Bsa01 and Pst1 sites, respectively. The mutation in exon 7 creates a restriction site for Mwo1.

Statistical Analysis

Data are shown as mean and SD. Student's unpaired *t*-tests were used to compare means of two groups. Differences among three-group means were estimated by the Kruskal–Wallis test. The chi-square test was used to compare frequencies. A p value < 0.05 was considered statistically significant.

Table 1. Subjects

Glucose tolerance	Sex (M/F)	Age (yr)	Body mass index (kg m^{-2})
Normal	139/81	55.8 ± 8.5	23.8 ± 3.4
Type 2 diabetes	118/118	56.4 ± 13.7	24.6 ± 4.9

Mean ± SD.

Results

The T761T substitution in exon 22 of the SUR1 gene was not found in either diabetic patients or controls. However, two other sites were highly polymorphic in the Japanese population; allelic frequencies of the exon 7 variant and the intron 24 variant were 0.43 and 0.54, respectively. Both the exon 7 variant and the intron 24 variant were present at the similar frequency in diabetic patients and non-diabetic subjects (Tables 2 and 3). No significant association was seen between the exon 7 variant or the intron 24 variant and BMI in either group.

We analysed the association of the polymorphism with family history of diabetes in first-degree relatives of patients (Table 4). Among the 231 patients from whom family history was available, a positive family history of diabetes was found in 64 %. Patients homozygous for the −3C allele of intron 24 had a significantly higher ratio of positive:negative family history than patients homozygous for the −3T allele or heterozygous for the substitution ($p = 0.03$, uncorrected for multiple comparisons), although there was no significant association between the genotypes as a whole and positive family history. Family history of diabetes was not associated with the exon 7 polymorphism.

Discussion

Studies on sulphonylurea receptor genes in Caucasian populations have shown a significant association of the C→T substitution in exon 22, but not the T→G substitution (S1370A) in exon 7, with Type 2 diabetes and obesity.[8,9] Linkage disequilibrium with a functionally relevant nearby mutation is a likely mechanism to explain the association of exon 22 polymorphism with Type 2 diabetes and obesity, because the C→T substitution does not result in amino acid change. Furthermore, a modest association has been reported between the T→C change in intron 24 and Type 2 diabetes or morbid obesity.[9] However, the clinical significance of the intron 24 polymorphism remains controversial. In the present study, the exon 22 variant was not found either in non-diabetic subjects or in patients with diabetes, in accordance with a previous report showing that the variant was extremely rare in Asian populations,[8] whereas the T→C substitution in intron 24 and S1370A substitution in exon 7 were common in Japanese. The absence of the exon 22 variant provided an opportunity to analyse the possible role of polymorphisms in intron 24 and exon 7 independently of the genotype of exon 22.

When comparing the allelic frequencies between the diabetes group and the control group, no significant difference was obtained in the exon 7 variant or the intron 24 substitution. Hani *et al.*[9] showed that homozygous carriers of the intron 24 −3C allele had a more severe form of obesity in France. However, in this study, no significant association was seen between either variant and obesity. This discrepancy may be attributable

Table 2. Glucose tolerance, body mass index (BMI), and polymorphism in exon 7 of the SUR1 gene

Glucose tolerance	Codon 1370 in exon 7			G allele frequency
	T/T	T/G	G/G	
Normal	81 (37 %)	99 (45 %)	40 (18 %)	0.41
BMI (kg m^{-2}) $<$ 22	23 (32)	37 (51)	12 (17)	0.42
22–26.4	38 (39)	40 (41)	19 (20)	0.40
$\geq$ 26.4	20 (39)	22 (43)	9 (18)	0.39
Type 2 diabetes	77 (33)	115 (48)	44 (19)	0.43
BMI (kg m^{-2}) $<$ 22	25 (33)	33 (43)	18 (24)	0.45
2–26.4	29 (33)	42 (47)	18 (20)	0.44
$\geq$ 26.4	22 (31)	40 (56)	9 (13)	0.41

Table 3. Glucose tolerance, body mass index (BMI) and T→C change at –3 of the intron 24 of the SUR1 gene

Glucose tolerance	–3 of intron 24			–3C allele frequency
	–3T/–3T	–3T/–3C	–3C/–3C	
Normal	47 (21 %)	113 (51 %)	60 (27 %)	0.53
BMI (kg m^{-2}) $<$ 22	17 (24)	40 (56)	15 (21)	0.49
22–26.4	20 (21)	48 (49)	29 (30)	0.55
$\geq$ 26.4	10 (20)	25 (49)	16 (31)	0.56
Type 2 diabetes	50 (21)	119 (51)	67 (28)	0.54
BMI (kg m^{-2}) $<$ 22	13 (17)	36 (47)	27 (36)	0.59
22–26.4	25 (28)	39 (44)	25 (28)	0.50
$\geq$ 26.4	12 (17)	44 (62)	15 (21)	0.52

Table 4. Polymorphisms of the SUR1 gene and family history of diabetes in first degree relatives of subjects with Type 2 diabetes

Nucleotide	History of diabetes in 1st degree relatives
Intron 24	
T/T	31/50 (62 %)
T/C	67/115 (58)
C/C	49/66 (74)[a]
Exon 7	
T/T	46/74 (62)
T/G	72/114 (63)
G/G	29/43 (67)
Total	147/231 (64)

[a] $p = 0.03$ vs T/T plus T/C.

to different environmental factors and genetic background.

The intron 24 –3C allele is more common in US (Utah) and UK subjects than in other Northern European subjects.[8,9] The former groups had a positive family history of diabetes, whereas the latter were randomly chosen. In our study, there was a suggestion that homozygotes for –3C allele had higher frequency of family history.

In conclusion, the polymorphisms we examined (exon 7 variant and intron 24 variant) are not major determinants of diabetes and obesity in the Japanese population.

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